

Molecular Epidemiology Study of a Suspected Community Cluster of Childhood Cancers

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We investigated the report of a community cluster of cancers in 33 children, which included two siblings known to have dominantly inherited Li-Fraumeni syndrome and a germline p53 mutation. After defining criteria for inclusion in the cluster, the 12 eligible childhood cancer probands diagnosed between 1980 and 1989 were not excessive (expected, ten cases). The corresponding childhood cancer mortality rates for the community fluctuated between 1950 and 1989 and were not increased overall. However, three additional probands had family histories of childhood cancer that suggested a

forme fruste of Li-Fraumeni syndrome. The epidemiological data suggested a geographic cluster of this rare hereditary disorder, but absence of germline p53 mutation in the three other multicase families indicates genetic heterogeneity. Laboratory studies can assist analyses of suspected clusters, although investigations of geographic clusters of hereditary cancers raise complex issues of confidentiality and protection of affected individuals, their families, and the community. *Med. Pediatr. Oncol.* 28:243–247.

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Key words: familial cancers; germline p53 mutations; time-space clusters; confidentiality and genetics

INTRODUCTION

Many reports have described community clustering of childhood cancers, particularly leukemias and lymphomas [1–9]. Causes of aggregates of childhood cancer have rarely been found, although occupational exposures and other environmental factors have helped explain geographic clustering of certain adult-onset cancers [10–12]. When an apparent time-space cluster of pediatric neoplasms was first described to us, no etiologic hypothesis was evident. Soon thereafter, one family in the cluster was discovered through another study to have Li-Fraumeni syndrome and a germline p53 mutation. The finding called attention to features of this dominantly inherited disorder among other families in the reported cluster, and additional studies were initiated.

MATERIALS AND METHODS

In 1989, we were notified of a possible cluster of childhood cancers in a rural U.S. county (community X; total population according to the 1980 census of the U.S. population, 32,000) [13]. Our informant had compiled a list of childhood cancer cases in the community. Her extensive inquiries regarding potential causes of the suspected cluster yielded no testable etiologic hypothesis. The uncertain results of prior studies of childhood cancer clusters were reported to the informant, and no further investigations were recommended at that time.

In unrelated studies, we had described dominantly in-

herited Li-Fraumeni syndrome of early-onset breast cancer and diverse childhood cancers, including sarcomas, brain tumors, acute leukemia, and adrenocortical carcinoma [14]. Five Li-Fraumeni families were found in 1990 to have inherited mutations in the p53 tumor suppressor gene [15,16]. One of these families was incidentally noted to reside in community X. When additional inquiries suggested features of Li-Fraumeni syndrome in other families in the community cancer cluster, a formal study was launched.

We sought to ascertain all childhood cancer cases in the community through inquiries of all local physicians, community hospitals, and the three regional medical centers to which children with cancer were usually referred. To examine the suspected cluster, criteria were defined for inclusion of individual cancer cases. Eligible subjects were children who developed a histologically diagnosed cancer at age 14 years and under, 1980 through 1989, while residing in the community. The expected number

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Received 6 November 1995; Accepted 11 April 1996

of cancer cases in the community was estimated by applying the childhood cancer incidence rates (129/million children/year) from the Surveillance, Epidemiology, and End Results (SEER) Program to the approximately 7,600 children in the community during the study interval [17]. The expected numbers of childhood cancer, by tumor type, were determined in the same manner. In addition, childhood cancer mortality rates for community X were obtained from computer files of cancer mortality by U.S. counties generated by the Division of Cancer, Epidemiology, and Genetics, National Cancer Institute [18,19]. These figures were compared with corresponding expected numbers based on age-specific national cancer death rates for each of the 4 decades between 1950 and 1989.

The parents of the probands in the cluster were interviewed by telephone to obtain a detailed family pedigree and history of cancer. Consent was obtained to document incident cancers through clinical records, pathology reports, and death certificates. In addition to the Li-Fraumeni family with a previously identified germline p53 mutation, other probands in the cluster and their relatives were found to have the types of childhood cancer featured in the syndrome. Specimens of whole blood were obtained from one affected child in each multicase family for germline p53 analysis. Genomic DNA was extracted and used for direct sequencing of all 11 exons of the p53 gene by previously described methods [20,21].

RESULTS

A total of 33 children in community X were reported to have cancer by local physicians, community hospitals, regional medical centers, and the informant who called attention to the cluster. However, only 12 patients fulfilled the criteria for inclusion in the cluster analysis. The remaining 21 were excluded for diverse reasons: date of diagnosis other than 1980–1989 (ten cases), residence outside the community at cancer diagnosis (five), over age 14 at diagnosis (four), and benign disease (two children). The 12 probands do not significantly exceed the ten cases expected during the decade of study (Table I). Five children developed brain tumors (1.9 expected), and three developed sarcomas (1.1 expected). The excess of these cancers is based on small numbers. In addition, selection bias exists because the present study was initiated after the finding of these tumors in three children in family 1 with Li-Fraumeni syndrome and in probands of families 2–4 (Table II).

Mortality data show that in the last four decades (1950 and 1989) cancer was the underlying cause of death at ages 0–14 years in 23 residents of the community (expected based on national rates, 23 deaths; RR = 1.0). The corresponding observed (O):expected (E) mortality figures for individual decades are 8(O):7(E) deaths in

TABLE I. Observed and Expected Numbers of Patients Diagnosed With Cancer at Ages 0–14 Years in Residents of Community X, by Tumor Type, 1980–1989

	Observed	Expected
Brain tumor	5	1.9
Sarcoma	3 ^a	1.1
Acute lymphocytic leukemia	2	2.2
Non-Hodgkin's lymphoma	1	0.7
Other ^b	1	4.1
All cancers	12	10.0

^aOne patient had two primary sarcomas, but only the first was counted in the analysis.

^bHepatoblastoma.

TABLE II. Cancers in Four Families in the Community Cluster

Family No.	Tumor type (relationship to proband, and age in years at diagnosis)	
	Proband	Affected relatives
1	Soft tissue sarcoma and osteosarcoma (1,8) ^a	Brain tumor (sib, 5) ^a Brain (maternal cousin, 12) Breast (mother, 30) Breast (maternal aunt, 28)
2	Rhabdomyosarcoma (2) ^a	Brain (cousin, 1) ^a
3	Soft-tissue sarcoma (11) ^a	Neuroblastoma (distant maternal cousin, 3) Acute leukemia (distant paternal cousin, 10) Breast (paternal grandmother, 69)
4	Brain tumor (13) ^a	Soft tissue sarcoma (maternal cousin, 11) Brain (paternal cousin, 14) Brain (paternal grand-uncle, 55)

^aChildhood cancers among residents of community X.

1950–1959, 2:8 in 1960–1969, 8:5 in 1970–1979, and 5:3 in 1980–1989. One of two excess cancer deaths during the 1980–1989 interval occurred in the Li-Fraumeni family with the germline p53 mutation.

Family histories of the 12 study probands revealed four familial aggregates of childhood cancers (Table II). Childhood cancer in the proband and a sibling (family 1) and early-onset breast cancer in their mother and maternal aunt are attributable to a germline p53 mutation [16]. Family 2 has a pair of first cousins with rhabdomyosarcoma and brain tumor, respectively. In family 3, the proband with soft tissue sarcoma has a distant maternal relative with childhood neuroblastoma, a distant paternal relative with childhood leukemia, and a paternal grandmother (age 64 years) with breast cancer. The proband and two distant paternal relatives in family 4 developed brain tumors without evidence of neurofibromatosis, and a distant maternal relative had a childhood soft tissue sarcoma.

Nearly all cancers documented in 15 patients in fami-

TABLE III. Cancer Occurrence in Four Families, by Age at Diagnosis and Tumor Type

Tumor type	Age at diagnosis (yrs)			Total
	0–14	15–30	>30	
Sarcoma	4 ^a	—	—	4
Breast cancer	—	2	1	3
Brain tumor	5	—	1	6
Other ^b	2	—	—	2
All types	11	2	2	15

^aOne patient had 2 primary sarcomas, but only the first was counted in the analysis.

^bAcute leukemia, neuroblastoma (1 each).

lies 1–4 are featured in Li-Fraumeni syndrome (Table III). There were 11 affected children: four children with sarcomas (one with multiple sarcomas), five with brain tumors, and one each with neuroblastoma and acute leukemia. Except for neuroblastoma, the other childhood cancers and the two early-onset breast cancers are components of the syndrome. However, only family 1 has classical Li-Fraumeni syndrome and an inherited p53 mutation [16]. DNA sequence analyses of all exons of the p53 gene failed to reveal germline mutations in families 2–4. Moreover, childhood cancers in families 3 and 4 occurred among distant relatives in both parental lines; the pattern is inconsistent with dominant inheritance and high penetrance. In family 3, the three cases of brain tumors suggest inherited site-specific cancer susceptibility, rather than germline p53 mutation [16]. These clinical and molecular findings suggest genetic heterogeneity among the four families.

The families were not aware of any unusual exposures to environmental carcinogens. Inquiries about carcinogens in community X revealed reports of chemical dumps, but there is no clear evidence that chemicals in the environment induce diverse forms of childhood cancers [7,8]. Families 1, 2, and 4 have resided in community X for many generations, whereas family 3 moved there in 1965. Among the four multicase families, only six affected children ever resided in community X (Table II); cancers in the other five affected children in families 1–4 cannot be attributed to environmental factors in the community. The families do not have blood relationships to one another and are not linked by close social contacts, shared parental employment, or proximity of places of residence within the community.

DISCUSSION

Reports of time-space clusters of childhood cancers have stimulated much scientific interest and public concern regarding the possible influence of environmental carcinogens, such as viruses, chemicals, and radiation [9–12]. To date, most epidemiological and statistical investigations have yielded inconclusive results [1–8]. A

number of reports conclude that clusters may be simply a result of chance events. Recently, studies of childhood leukemia in Great Britain have suggested a tendency to cluster in newly constructed towns with population immigration, consistent with the possible role of an infectious agent [22]; however, no such pattern has been reported in the United States or other countries. To our knowledge, no time-space clusters of childhood cancer have been linked to familial or genetic susceptibility among multiple unrelated kindreds, as noted in the present investigation.

No etiologic hypothesis was available to explain the reported cancer cluster when community X was first brought to our attention. Soon thereafter, one of the families in the cluster was found through an unrelated study to have Li-Fraumeni syndrome and a germline p53 mutation [16]. The observation prompted additional inquiries regarding the family histories of the other children in the cluster, and three additional multicase families were found. The predominant cancers in these three families are childhood sarcomas and brain tumors featured in Li-Fraumeni syndrome. However, these families do not have a germline p53 mutation. Moreover, childhood cancers in five of their relatives who reside elsewhere cannot be attributed to environmental carcinogens in community X.

Criteria were needed to define the eligibility for inclusion in the community cluster, which resulted in the elimination of many cases that brought the community to the attention of our informant. Only 12 of the original 33 cases were eligible for the cluster analysis of the 1980–1989 interval, and no excess childhood cancer incidence or mortality was detected. Eligible cases were sought in the community and regional medical facilities. Underascertainment of cases is unlikely because few primary care facilities and referral centers serve this rural area. Extending the interval of study to the prior decade was not feasible because of incomplete case ascertainment. After the close of the present study, a lymphoma developed in an additional child who has a family history of childhood cancer, but no new childhood cancers have occurred in families 1–4. Prospective observation has been underutilized in previous cluster studies due to high cost of follow-up and long study duration. A collaborative follow-up study of all previously reported childhood cancer clusters might be of interest [23].

This report underwent multiple revisions because of concerns about the confidentiality of genetic data in published studies [24]. Early drafts contained the pedigrees of the four multicase families. Identifying features of the community were also provided, and our informant was acknowledged by name in a footnote. These items were subsequently removed from the manuscript because of new guidelines from the Office for Protection from Research Risks (OPRR) of NIH regarding publication of

human genetics research. These new guidelines remarked on the need to consider whether the reported pedigrees are essential to the study [24,25]. OPRR noted that risks to study subjects might result from publicity that exposes the families, and in this instance an entire community, to adverse social, psychological, and economic effects. OPRR stated that publication of genetic data that can identify study participants is permissible when the information is essential and when written informed consent has been obtained from the subjects.

We submitted this manuscript without the pedigree and other identifying data on the affected families and their community. However, one reviewer stated that, "T(t)he genetics of cancer and of normal human biology have been immensely illuminated by the publication and analysis of pedigrees. The reader is greatly handicapped by their absence. I consider them essential to this report." It is unclear why the pedigrees are essential in this study, since no time-space clustering was found, and the multiple cancers in families 2–4 are unexplained. On the other hand, several reasons exist for not showing the pedigrees [24,26,27]. First, following OPRR recommendations to request consent for publication of the pedigrees might cause distress to the families, and it is unclear which family members need to consent. Second, an entire community is at risk of being stigmatized, and obtaining consent from an entire community does not seem possible. Last, it is unclear that the need to provide pedigrees supercedes the rights of the families and the community to privacy. In recognition of the perspective of the reviewer, we added Table II to show only the affected relatives and their relationship to the proband.

With increasing concerns among ethicists, investigators, and the public about adverse consequences of intentional or unintended disclosure of genetic information, publications of genetic results should be sensitive to issues regarding the protection and privacy of research subjects [24,26–28].

CONCLUSIONS

In conclusion, no evidence was found for time-space clustering of childhood cancers in community X. However, the cluster included four familial aggregates of childhood cancers, including one family with known Li-Fraumeni syndrome and a germline p53 mutation. In contrast, the three other families with some features of the syndrome had no detectable germline p53 mutation. Available evidence suggests chance association within the reported time-space cluster and some multicase familial clusters, but prospective observation of both the community and families might yield more information.

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